Treatment of human multiple myeloma cell lines *in vitro* using EZH2 inhibitors GSK126 and EPZ-6438

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Background

Multiple myeloma (MM) is the second most common hematopoietic neoplasm, accounting for 22,000 new cases in the U.S. each year and 2% of all cancer deaths. MM is characterized by the proliferation of malignant plasma cells in the bone marrow, and is a mostly incurable disease due to the genetic heterogeneity between MM patients, as well as within a single patient due to the presence of subclonal tumor populations. Addressing this heterogeneity requires an individualized treatment approach with a wide array of drugs that have different targets. Previously in the Van Ness laboratory, it was demonstrated that the polycomb group protein enhancer of zeste homolog 2 (EZH2) acts as an oncogene in multiple myeloma[1]. EZH2 is the catalytic subunit of the polycomb repressive complex 2 (PRC2), repressing gene expression via the methylation of histone H3 on lysine 27 (H3K27)[2]. The epigenetic inactivation of tumor suppressor genes, presumably via EZH2, has been associated with a poor prognosis in multiple myeloma[3]. Additionally, there is an enrichment of H3K27me3 at genes that are commonly underexpressed in multiple myeloma cells, including known EZH2 targets, which could likely be due to increased EZH2 activity[4]. Overexpression of EZH2, as well as EZH2 mutations, have been found in other cancers, such as prostate and breast cancer and lymphoma[2,5], leading to the development of several novel EZH2 inhibitors. Two of these inhibitors are GSK126 (GlaxoSmith Kline) and EPZ-6438 (Epizyme), which are both highly selective, competitive small-molecule inhibitors of EZH2 methyltransferase activity. In recent studies, GSK126 and EPZ-6438 both inhibited the proliferation of EZH2 mutant diffuse large B-cell lymphoma (DLBCL) cells and inhibited the growth of EZH2 mutant DLBCL xenografts in mice[2,6]. Because of these recent developments, we **hypothesize** that EZH2 inhibition will reduce viability of human MM cell lines (HMCLs) *in vitro*, and that the differences seen in EZH2 expression levels would correlate to sensitivity to EZH2 inhibition.

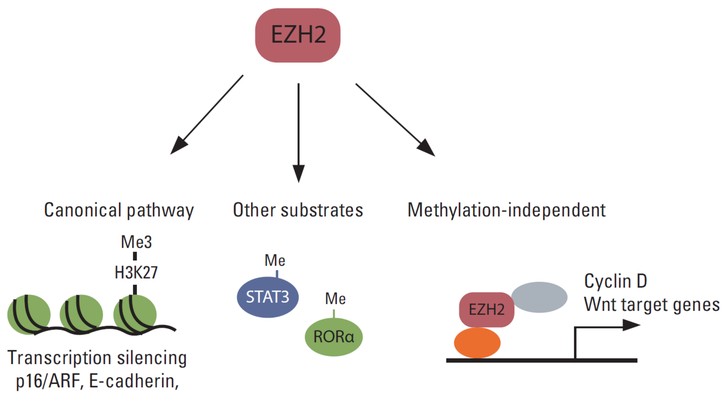

Figure 1[5]: The functions of EZH2 in cancer. EZH2 silences tumor suppressor genes via the methylation of histone H3 on lysine 27 (H3K27). EZH2 also methylates substrates other than H3K27, and has a methylation-independent mechanism of action.

Methods

GSK126 and EPZ-6438 were used in single-agent dose response viability assays with human multiple myeloma cell lines (HMCLs) containing the lowest and highest levels of EZH2 expression. By determining the IC_{50}s of GSK126 and EPZ-6438 in these cell lines, it could be determined if there were differences in sensitivity to EZH2 inhibition that were dependent on EZH2 expression. Additionally, it was determined whether or not EZH2 inhibition occurred in these cell lines by extracting histones in HMCLs after treatment with GSK126 or EPZ-6438, and performing a Western Blot with antibodies for total H3, and mono-, di-, and tri-methylation status. Successful EZH2 inhibition is indicated by increased demethylation on histone H3. Finally, GSK126 and EPZ-6438 were used in double-agent dose response viability assays with other multiple myeloma drugs, including the proteasome inhibitors Bortezomib and Oprozomib, and the histone deacetylase inhibitor Panobinostat, to observe if these drugs worked synergistically.

Results

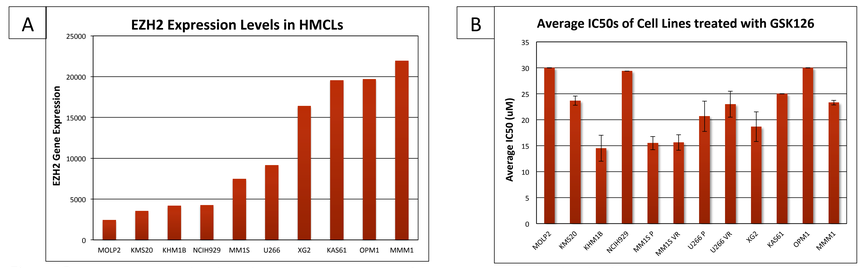

Figure 2: EZH2 expression levels and sensitivity to GSK126. A) EZH2 gene expression levels of HMCLs. EZH2 expression was determined by gene expression profiling (Affymetrix) by Dr. Michael Kuehl (NCI) and Dr. Jonathan Keats (TGI, Phoenix). **B)** IC50s of human multiple myeloma cell lines with GSK126. HMCLs presented from left to right order from low to high EZH2 expression. For KMS20 n=6, for KHM1B n=2, for NCIH929 n=4, for MM1S P and VR n=4, for U266 P and VR n=3, for XG2 n=3, for MMM1 n=5. Error bars represent standard error of the mean. MOLP2, KAS61, OPM1 all had n=1 so SEM could not be calculated.

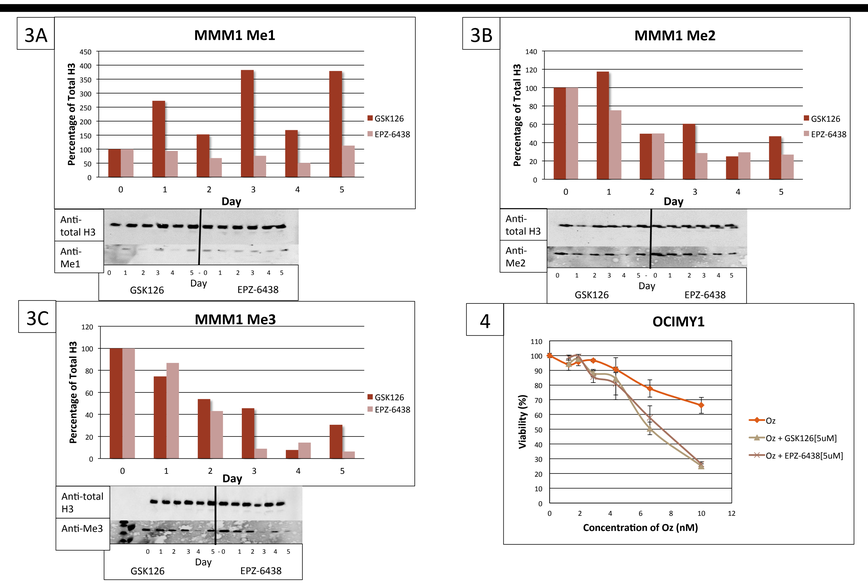

Figure 3: Methylation status of Histone H3 after EZH2 inhibition. Quantification of the mono-, di-, and tri-methyl groups on Histone H3 relative to total H3 levels after 0-5 days of treatment with 5 μM GSK126 or EPZ-6438.

Figure 4: EZH2 inhibitors in combination with other drugs. HMCL OCIMY1 after 48 hours of treatment with 0-10 nM of the proteasome inhibitor Oz, 0-10 nM of Oz with 5 uM GSK126, and 0-10 nM of Oz with 5 uM EPZ-6438.

Conclusions

- GSK126 reduces cell viability, with IC_{50}s of 15-30 μM, independent of EZH2 expression level, while EPZ-6438 does not reduce cell viability on its own.
- Treatment with 5 μM GSK126 or EPZ-6438 effectively reduces the levels of di-methyl and tri-methyl groups on histone H3, but not levels of mono-methyl. This is most likely due to the low levels of mono-methyl groups that are present in the control. The demethylation effect is maximal after 4-5 days.
- Since GSK126 and EPZ-6438 both cause demethylation on histone H3, they are successfully inhibiting EZH2. However, this EZH2 inhibition occurs at a concentration (5 μM) that is much lower than the concentrations that induced apoptosis. This indicates that GSK126's ability to induce apoptosis is due to an off-target effect. This effect is absent in EPZ-6438, which is why EPZ-6438 does not reduce viability.
- In some cell lines, non-cytotoxic doses of EZH2 inhibitors work synergistically with proteasome inhibitors (Oz) and HDAC inhibitors (Panobinostat), causing more kill than Oz or Panobinostat on their own.

Future Directions

- Continue testing EZH2 inhibitors with proteasome inhibitors and HDAC inhibitors, including testing the effectiveness of pre-treating cells with EZH2 inhibitors and then treating with other classes of drugs.
- Determine which doses of GSK126 and EPZ-6438 achieve maximal EZH2 inhibition.
- Explore mechanisms of synergy between EZH2 inhibitors and proteasome/HDAC inhibitors.
- Determine if any HMCLs contain EZH2 mutations by cDNA sequencing with EZH2, since these cell lines may experience an apoptotic effect from EZH2 inhibition unlike wild type EZH2 HMCLs. They would also be much more sensitive to GSK126 and EPZ-6438.

References

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